JPRS-JST-90-016
20 MARCH 1990

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JPRS Report

Science & Technology

Japan

1989 ERATO SYMPOSIA

JPRS-JST-90-016
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SCIENCE & TECHNOLOGY
JAPAN

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43075003 Tokyo 1989 ERATO SYMPOSIA in English Vol 1, 30 Nov 89 pp 1-89; Vol 2, 1 Dec 89, pp 1-65; Vol 3, 7 Dec 89 pp 1-111; Vol 4, 8 Dec 89 pp 1-76

[Selected abstracts of papers presented at the 89 ERATO Symposia held 30 Nov-8 Dec 89 in Tokyo and sponsored by the Research Development Corporation of Japan. The papers published in these four volumes constitute an update on ERATO research projects.]

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Vol. 4

Mizutani Plant Ecochemicals Project

Junya Mizutani

Among organisms higher plants cannot change the location of their existence, but they adapt themselves to the given environment, and are equipped with defensive and offensive mechanisms to protect themselves and promote the prosperity of their species. These defence mechanisms have been acquired during the long process of evolution, and here chemical substances (ecochemicals) play an important role especially in wild plants.

We have already isolated more than 100 ecochemicals that are synthesized in the roots, leaves or other part of plants and released to the immediate environment to influence animals, insects, bacteria and fungi as well as competitive plants. This project will greatly enhance our efforts to identify and isolate physiologically active biorational ecochemicals that may act as model compounds for ecologically safe agriculture pesticides, food processing and pharmacology.

Subjects of research groups

1. Formation Mechanisms Research Group

   The biosynthesis and metabolism of ecochemicals and their various regulatory mechanisms are being studied.

2. Allelochemicals Research Group

   The evolution and role of economicals in the interactions of wild plants with other plants or microoganisms are being studied as well as their transformation into new physiologically active substances through the action of microorganisms.

3. Plant-Animal Interactions Research Group

Plant ecochemicals, such as insecticides, repelants and antifeedants are being studied by developing new bioassay systems which clarify the defense mechnisms of plants against animals.

Approach to Mechanisms of Ultraweak Biophoton Phenomena

Project Director

Humio Inaba

The Biochemical Information Group is pursuing measurement and characterization of biophoton phenomena from biological tissues and cells in vivo primarily without external or artificial stimulation, and analysis of light emitting species and their reaction processes from the biochemical point of view. Based on knowledge obtained by such measurement and analysis, this group intends to search for biophoton emission mechanisms and to explore the possibility of applying this technology and method biochemically.

Current research efforts of our group include detection of ultraweak biophoton emission in the near-infrared region from biochemically generated singlet molecular oxygen, which is believed to be one of main sources of biophoton emission. By using the highly sensitive near-infrared emission spectrometer, we have developed new reagents generating singlet molecular oxygen effectively in an organic solvent and have proved also that cation radicals react with superoxide anion radical to give singlet molecular oxygen on their annihilation. This near-infrared emission spectrometer has allowed us to measure the quenching rate constants of many important, additive reagents for biochemical investigation, such as NaN_3 and superoxide dismutase and also those of some sensitizing chemiluminescent reagents (*Cypridina* luciferin analogues and luminol).

Our group is also continuing the biochemical study of the mechanism of biophoton emission from Japanese sea urchin eggs in fertilization. In fertilization of sea urchin eggs with seminal fluid, ovoperoxidase is secreted from the eggs causing a hardening of the fertlization membrane,

the formation of cross-linkages between tyrosine residues in the membrane protein. The biophoton emission is generated directly by one-electron oxidation and its annihilation of tyrosine or bityrosine. Using a newly developed, very high-sensitivity computer-controlled filter spectral analyzer system in the ultraviolet-visible region, the emissive oxidation mechanism was demonstrated by means of the electric oxidation of tyrosine.

The ultraweak biophoton emission from *Drosophila melanogaster* administered mutagens or carcinogens was studied. The emission intensity was evidently enhanced by the mutagen and carcinogen administration. *Drosophila melanogaster* fed with polycyclic aromatic hydrocarbon (PAH) quinones showed a linear relation between the biophoton emission intensity and the mutation frequency in the *Drosophila* wing spot test. The intensity from *Drosophila melanogaster* fed with the bracken fern power containing carcinogen increased in proportion with the dose of the carcinogenic powder. However, it was decreased by addition of quenchers and scavengers, suggesting that free radicals and active oxygen species participated in this biophoton emission phenomenon. Phosphatidylcholine hydroperoxide (PCOOH) concentration was increased in proportion to the intensity. It was clear that much lipid peroxide is accumulated in the process of mutation on *Drosophila melanogaster*

The effects of dietary restriction on superoxide production in macrophage are also studied by utilizing ultraweak photon emission detection. It has not been clarified whether dietary restriction alters macrophage functions, although the augmentation of T cell functions by dietary restriction is well known. By using a chemiluminescent probe, 2-methyl-6-(p-methoxy-phenyl)-3,7-dihydroimidazo 1,2-a phydrazin-3-one (MCLA), it was shown that 40%-dietary restriction in 9-week-old male C3H/He mice caused an augmentation of one of the major macrophage functions, the generation of

superoxide anion (O_2) in proteose peptone-elicited peritoneal macrophages (MPs). It was further clarified that the increase in O_2 generation in opsonized zymosan (OZ)-stimulated phagocytosing MPs by dietary restriction was not inhibited by the PKC inhibitor (H-7) and that the increase was completely abolished when the CaM antagonist (W-7) was added. These results clearly showed that the augmentation of the O_2 generation in phagocytosing MPs by dietary restriction was due to the increased activity of CaM in the MPs, and that PKC was not involved in the augmentation. It is thought that one of the major factors in the reduced incidence of tumor and infection in diet-restricted animals is the augmentation of O_2 generation in MPs.

Chemiluminescence of amino acids oxidized by singlet molecular oxygen was measured and the oxygeneration products of methiomine and histidine were analyzed by chemiluminescence - high performance liquid chromatography (CL-HPLC). They showed an intense chemiluminescence in the visible wavelength region. Oxidized methionine, arginine and lysine also showed strong chemiluminescence when reacted with a luminol-cytochrome c solution. By using a CL-HPLC, several chemiluminescent chromatographic peaks could be found for oxidized histidine and also for oxidized methionine. The sources of chemiluminescence of oxidized histidine is recognized to be excited carbonyl compounds, endoperoxidic and hydroperoxidic derivatives, and a secondary products such as hydrogen peroxide. That of oxidized mechionine is susceptible to be methionine peroxide and hydrogen peroxide. CL-HPLC appears to be useful in analysis of the oxidized amino acids.

High Sensitivity Measurement Technology for Biophoton Research

R.Q. Scott

Masahirc Toida

The Measurement Technology Group is actively involved in the development of a variety of new instrumentation which will allow us to better detect, analyse and study the phenomenon of biophoton emission. A number of new and highly sensitive devices have been developed and these are the mainstays of our colleagues in the other two groups. We have, since the inception of our project, developed what we believe to be the most highly sensitive and reproducible biophoton emission measurement techniques in the world, based primarily on the photon counting technique.

The activities of the Measurement Technology Group are by no means restricted to the development of instrumentation, but include a strong effort in fundamental and applied research into the biophysics of biophoton emission and its characterization. This research effort, combined with our new instrumentation and techniques, has led to important discoveries and advances in a number of areas.

We have, for example, detected a very weak photon emission in human breath which differs markedly between the states of rest, moderate exercise and heavy exercise. We are now investigating the potential clinical implications of these findings.

A very important and difficult problem in biophoton research has been to detect very weak light emission in the near infrared region where certain species of oxygen, so vital to life processes, are known to emit. We have recently made remarkable advances in this area through development of a PIN/Charge Integrating Amplification (P/CIA) detection technology.

Ultimately, the fundamental physical characterization of biophoton emission requires the capability to measure the spectra of very weak light with high resolution. We have developed a two-dimensional photon counting based transmission spectroscopy system to the extent that we now routinely produce the only high resolution spectra of ultraweak sources in the world. Using this instrument, we have published in collaboration with the Information Processing Group significant contribution in the field of photosynthesis research. Our technique of ultraweak spectroscopy has also allowed us to detect for the first time in a biological system a second order emission process, a finding of fundamental significance in biophysics.

By incorporating our most sensitive photon detection systems, an advanced Filter Spectral Analyzer System (FISAS) has been constructed. With this instrument the first clinical spectra of light emission from human sputum has been measured.

Having proven the effectiveness of our systems approach, we are building a second generation spectroscope. A new approach, incorporating an F 0.85 reflection grating is now undergoing tests, and appears to offer a significant advance.

At this point in time our basic instrumentation, in both photon counting and spectroscopy, has been perfected. In the time remaining to our project, we will endeavor to further increase our understanding of the significance of ultraweak photon emission from living organisms.

Dynamism of Biophoton Emission as a Source of Biological Information

Information Processing Group

Masashi Usa

Ultraweak biophoton emission can be considered a characteristic phenomenon observed in microscopically and macroscopically self-organized biological systems. From this point of view, the Information Processing Group is involved in multiple investigations of biophoton emission as a source of biological and vital information originating from a variety of living systems. In other words, analysis of biophysical and/or physiological information, given by the biophoton emission phenomena, in terms of synergetics and information processing is the central theme of our research group. On the basis of the knowledge thus derived, systematic studies of the relationships between characteristics of ultraweak biophoton emission and various biological phenomena and pursuit of an understanding of the inherent role and fundamental mechanisms of biophoton emission are also in progress.

In order to grasp the "meanings" and "raison d'etre" of biophoton emission and to exploit its "dynamisms", we have been performing a number of experiments and analyses. Here we report briefly the progress and results of our recent studies:

i) Two-dimensional pattern measurements and analyses of the effect of external stimuli (such as physical wounds) on ultraweak biophoton emission originating from a germinating soybean and the skin surface of a mouse.

ii) Simultaneous measurements and characterization of spontaneous ultraweak biophoton emission and bioelectric potentials originating from

a carrot callus and a mature plant system.

iii) High resolution spectral analysis of long-term delayed fluorescence generated from isolated spinach chloroplasts to approach an understanding of its origin and its kinetic properties in comparison with ultraweak biophoton emission. This work is being done in collaboration with the Measurement Technology Group.

iv) Synergetic study of weak photon emission (chemiluminescence) in nonequilibrium enzymatic open-reaction systems as dynamic models of formation in macroscopic order.

Epitaxial Growth of Metals with Strained Lattices

Atsushi Maeda

Basic Properties Group

Eu/Yb superlattice with bcc-Yb, high temperature phase, was successfully synthesized on Eu buffer layer by the molecular beam epitaxy technique. The bcc structure of Yb metal did not transform to an ambient temperature fcc phase in spite of its large thickness (at least 1500 Å). Therefore, it was first demonstrated that the bulk crystal film of high temperature phase could be fabricated using an epitaxial method. The Yb film with strained lattice was also epitaxially grown on cleaved NaCl surface by same method. This technique provides a general approach for the realizing of new materials with high temperature phase or metastable phase.

Synthesis of New Materials by Use of Surface Functional Groups

Yoshiyuki Nabata

Reactivities Group

Oxide surfaces were modified using metal carbonyl compounds by taking advantage of selective reactions between the metal carbonyl and surface functional groups. A dinuclear iron carbonyl cluster was attached for the first time to the surface of silica via chemical reaction between pre-attached surface HSi-groups and the iron carbonyl. Triiron dodecacarbonyl was also successfully attached for the first time to the surface of silica by a photochemical grafting reaction between surface hydroxyls and the iron carbonyl. In addition, osmium dodecacarbonyl was attached to the surface of silica via a photochemical grafting reaction in which the wavelength of the light used allowed control over the nuclearity of the attached osmium carbonyl formed.

These surface modification methods can be applied to the surface of silicon where this methodology is expected to be important in the design of molecular devices on semiconductor surfaces.

Formation of Oriented Organic Films by Molecular Beam Techniques

Shintarou Hattori

Functional Structure Group

It is of great technological importance to establish the method to align organic molecules having specific functions over solid surfaces. This study was undertaken to investigate the possibility of the molecular-beam deposition method for the purpose to prepare a highly-oriented, high-quality film of organic compound on a well-defined solid surfaces under UHV condition.

The optimal condition of molecular-beam deposition of a rather volatile organic compound was investigated by use of the computer simulation with Monte Carlo method, and a special type of K-cell was designed and constructed according to the result of the above investigation.

By use of the apparatus thus constructed, Cu-phthalocyanine films were formed on a clean and oxidized silicon surfaces to see the effect of the substrate surface. It was confirmed that the deposition of Cu-phthalocyanine can be made under UHV condition without causing contamination of the apparatus. The crystallinity of Cu-phthalocyanine film thus formed were found to be significantly different between the case of a clean silicon surface and that of an oxidized silicon surface, showing that the crystallinity and orientation of the film were strongly affected by the nature of the substrate surface.

Sakaki Quantum Wave Project

Exploration for New Fabrication Method and

Physics of Quantum Wave Structure

Project Director

Hiroyuki Sakaki

The evolution of the theory of quantum mechanics has made possible a deep understanding of the characteristics and behavior of electrons, atoms, molecules and crystal structures, resulting in a wide variety of applications. Though in quantum mechanics there is the wave-particle dualism as well as the uncertainty principle, these phenomena do not limit applications but, rather, foster wide ranging possibilities.

In spite of the fact that electrons in a semiconductor are often analyzed in terms of quantum mechanics, the behavior of electrons in devices with dimensions greater than 1000 angstroms can be thought of in terms of particles since the wavelengths involved are relatively small. Most existing semiconductor devices have sizes greater than 1000 angstroms ($1\mu m$ = 10,000A). The transistor, for instance, can be described in terms of the control of a current, just as gas flow in a pipe.

However, with modern nano-fabrication techniques, there is now the possibility to make semiconductor structures with atomic-scale features. At this level electrons loose (at least partially) their particle nature and behave like waves. They are, thus, quantized with energies restricted to a certain number of levels, depending on the wavelengths of the electrons in the structure, much as sound in an organ pipe is restricted by the pipe length.

The present project is studying quantum wave effects in advanced quantum microstructures which have hitherto been difficult to produce. These include such novel structures as quantum wires and boxes as well as highly heterogeneous layered structures containing metals and/or insulators. If the quantum effects in such systems can be fully understood and controlled, it may well become possible to create and exploit completely new families of applications which would reflect a new type of quantum electronics.

One part of this project is seeking a new materials technology which would make it possible to confine electrons in new dimensions — quantum wires and boxes — using microstructures with sizes ranging between 50 and 200 angstroms and controlled in both two or three dimensions. Since the preparation of such structures is still quite difficult, even using advanced lithographic techniques, several novel approaches are being studied.

A second part of this project is emphasizing both material technology and the properties of novel ultrathin layered structures which contain not only semicronductors but metals, insulators, and/or polymers. Once the method to prepare thse novel material systems has been established, they can possibly be used to make a new class of quantum wave devices.

This project is also seeking new ways to characterize, control, and exploit all of the above-mentioned quantum effects for the possible creation of applications which transcend the usual ways of conceiving electronics and devices. One possiblity is a novel electronic filter in which electrons of a particular wave-length would be strongly reflected by periodic arrays of quantum wires and/or boxes. Electrons confined in a wire structure should also exhibit novel motion in which there is no change in the propagation direction through scattering, an ideal material system for electrical conduction.

Another possible use is quantum box arrays used as a semiconductor laser material. Since electronic levels would be completed quantized and discrete, this system should act like a kind of "artificial atom" with a sharp optical spectrum. Such devices may open a whole new field of science and technology in which the quantum wave nature of electrons could be fully controlled and exploited.

Kunitake Molecular Architecture Project

Novel Functions through Self-organization of Molecular Materials.

Project Director

Toyoki Kunitake

The structural organizations of industrial materials such as metals and polymers are crucial to their performances and functions. The sophisticated functions observed in the living system such as energy transduction and information transfer are also directly associated with its intricate molecular organizations. It has to be noted, however, that there is a great gap in the extent of sophistication between the non-biological and biological organizations.

Recent rapid progresses in the biological science have opened ways to artificially construct molecular architectures which can mimic those of the living system. The biomembrane is formed by self-organization of component molecules (lipids and proteins) that is derived from their unique steric structures, and the lipid bilayer is the most essential structural organization.

In recent years, the preparation of synthetic bilayer membranes has been successfully achieved in Japan and other countries by using a large variety of novel organic compounds. These compounds are composed of hydrophilic head groups and hydrophobic hydrocarbon chains in an analogy to biolipid molecules.

Their particular structural features play an essential role in self-assemblage to bilayer embranes. This new synthetic approach in molecular architecture provides an exciting possibility to produce artificial

organization and lead to a new field of the chemical science.

In this project, the major emphasis is placed on the methodological development for self-organization of the above-mentioned types of organic molecules. The subsequent preparation of functionalized molecular architectures and elucidation of their physicochemical characteristics in the form of surface monolayers, LB films, aqueous bilayers and their composites are being conducted. A search is being made for molecular organizations which would possess particular electronic, magnetic, and chemical functions. Improvements of these functions will be attained by means of chemical modifications of simple assemblage and formation of multiply-composed organizations.

These studies should provide clues for the development of novel industrial materials that are equipped with sophisticated functions analogous to those of the biological organization and yet are characterized by stability and processability of synthetic materials.

The major laboratories of this project are set up at Kurume Research Park in Kyushu Island. The total staff number is 27, as of October 1, 1989, including project directer, administrative staff, researcher and technician. The whole research team is divided into three subgroups. The current activities of each group are described below.

1. Fundamental Design Group

A systematic study is being conducted on the structure and the property of self-assembling surface monolayers. Various monolayer forming compounds were newly synthesized in order to perform molecular recognition at the interface. Ultra sensitive spectrophotometry has been used to directly follow the chemical process on the water surface.

Novel microscopic techniques such as surface forces microscopy and

scanning tunneling microscopy were introduced along with more conventional electron and light microscopies. Their effective uses will facilitate structural elucidation of monolayers with the molecular resolution.

2. Functional Architecture Group

The major target of this group is the preparation of self-Organizing materials with novel electronic and magnetic properties. In particular, specific introduction of transition metal ions is being carried out by attaching ligand units to self-assembling compounds.

Molecularly-organized silicates have been developed, and anisotropic arrangements of metal chelates and iron oxides were achieved. Esr spectroscopy, electron microscopy and X-ray diffraction are used to study the structure of these materials.

3. Composite Architecture Group

Intricate functions such as observed in biomembranes cannot be produced by simple association of amphiphilic molecules alone. Hybrid materials prepared from two-dimensional molecular layers and one-dimensional polymer chains should provide a new type of the molecular composites. Two-dimensional molecular networks were obtained by this approach. Polymerization of bilayers and cast films is therefore under investigation. Preparation of ultra-thin, free-standing films based on stabilized monolayers and bilayers would give new permselective membranes.

Molecular Recognition by Surface Monolayers

Kazue Kurihara

Fundamental Design Group

Molecular recognition based on surface monolayers is important with regard to elucidation of biological cell functions and for development of new functionalized materials. We design and synthesized new monolayer-forming compounds which are capable of molecular recognition. New characterization methods were employed to follow the chemical processes at the interface.

A new type of ionophore monolayers were synthesized. A combination of the surface pressure-area isotherm and fluorescence microscope images is useful to examine the structure of monolayer formed. Ultra-sensitive reflection spectroscopy was used to monitor binding of alkali-metal ions to these monolayers.

Resorcinol-dodecanal cyclotetramer forms a stable monolayer. This monolayer bind various sugars and water-soluble polymers selectively. A chemical sensor for sugars was designed by using potentiometric response of electrodes modified by resorcinol monolayers.

Various long alkyl derivatives of Kemp's acid were synthesized. The structural unit of Kemp's acids forms dimers in these monolayers. Molecular recognition of nitrogen-containing heterocycles and amino acids was studied. FT-IR spectra suggest formation of the hydrogen bond between monolayers and substrates.

Two-Dimensional Polymer Network

Katsuhisa Kohyama

Composite Architecture Group

Ultra thin polymer films were prepared by the help of self-organizing compounds. These films are made of multi-layered two-dimensionally cross-linked polymer networks.

The individual layer appeared to thicknesses of about 100 Å. The multilayered films are stable and flexible, and show anisotropic mechanical properties due to in-plane crosslinking.

The present method would have a general applicability.

Yoshida Nano-Mechanism Project

- The Way to Nanometer Technology -

Shoichiro Yoshida

Project Director

1. The aim of Yoshida Nano-Mechanism Project

In our project, new scientific technologies in the fields of measurement, control and processing with nanometer precision are studied.

Though it is anticipated that the extention of some of the present technologies to the nanometer region will be difficult. Some of the obstacles come from the fact that the nanometer region is very close to atom or molecule size, so conventional measuring methods are no longer applicable and solids and liquids cannot be treated as uniformly continuous objects.

However possibilities of the breakthrough will be emerging through the studies focused on the physical actions and mechanical properties of matters in the nanometer region, because the phenomena of this region are derived from atom or molecule behavier.

Through the study of new measuring and processing method in which newly developed principles are applied, the essential technologies for constructing next generation high technology machine will be learnt.

But the important results are not the developed instruments and components. The technologies learnt in the building of the instruments and the understanding derived from using these instruments are important.

Our project is being carried out by three groupes, totaling 14 researchers now. The Basis Analyses Group and the Measurement and Control Group are located in Tsukuba, while the Processing Group is in

Tokyo in the facility of Nikon Corporation. Most of the researchers are coming from companies. This is the case of our project. Two researchers have changed their belonging from universities to our project. An overseas researcher is coming from Intel Corporation of the U.S.A.

2. Current Research Activities

2.1 Basic Analyses Group

(1) Scanning Tunneling Microscope

Since Binnig, et al., built their first instrument the scanning tunneling microscope (STM) has evolved rapidly. The STM is a novel surface analysis tool for real-space imaging of surface structure and composition with atomic resolution. The STM can measure the structure of any conducting surface up to several micrometers in area with nanometer vertical resolution as a result of improvements in the scanning method. Thus the study of the STM and the study using STM are very important and useful for our project.

For observing processed surfaces or biological specimens with sub-nm resolution, a scanning tunneling microscope-scanning electron microscope (SEM-STM) combined system has been developed. It has a wide range of magnifications from standard SEM magnifications to atomic scale magnifications. The SEM is also useful for finding a measurement area suitable for STM observation. The STM is installed inside the sample chamber of a commercial SEM. The STM sample and STM tip can be observed by the SEM. An STM image can be displayed on the SEM's CRT by flipping several switches. With this STM, we studied surfaces etched by glow discharged argon ions. Fig. 1 shows computer-processed STM images. We found that the roughness caused by etching saturates after 6 min of etching with a roughness about 1 nm.

We are interested in the etching process at the atomic level, i. e., how are atoms sputtered when a single ion bombards a surface. To study this more closely, an STM inside of an ultra high vacuum chamber (UHV-STM) is under construction. This UHV-STM will be combined with an ion beam processing machine which is currently being used by the processing group. Samples will be transferred from the etching chamber to that of the STM's without leaving vacuum. Surfaces processed by ion beams can be studied by the STM without contamination.

An attempt is being made to use an STM in industrial applications, e.g., VLSI fabrication process studies. The STM will be operated in air and at relatively long distances from the samples. We are building an STM which will be set on top of the wafer and capable of inspecting small areas on unbroken, patterned silicon wafers of up to 150 nm diameter. 3 micromanipulator probes will be used to provide voltage inputs to transistor structures on the wafer. So we will be able to observe the structure of the semiconductors in active condition.

And we developed another STM for the research of industrial application. This system allows nanometer tip positioning with a simple way to compensate for hysterisis and creep in piezoelectric actuator.

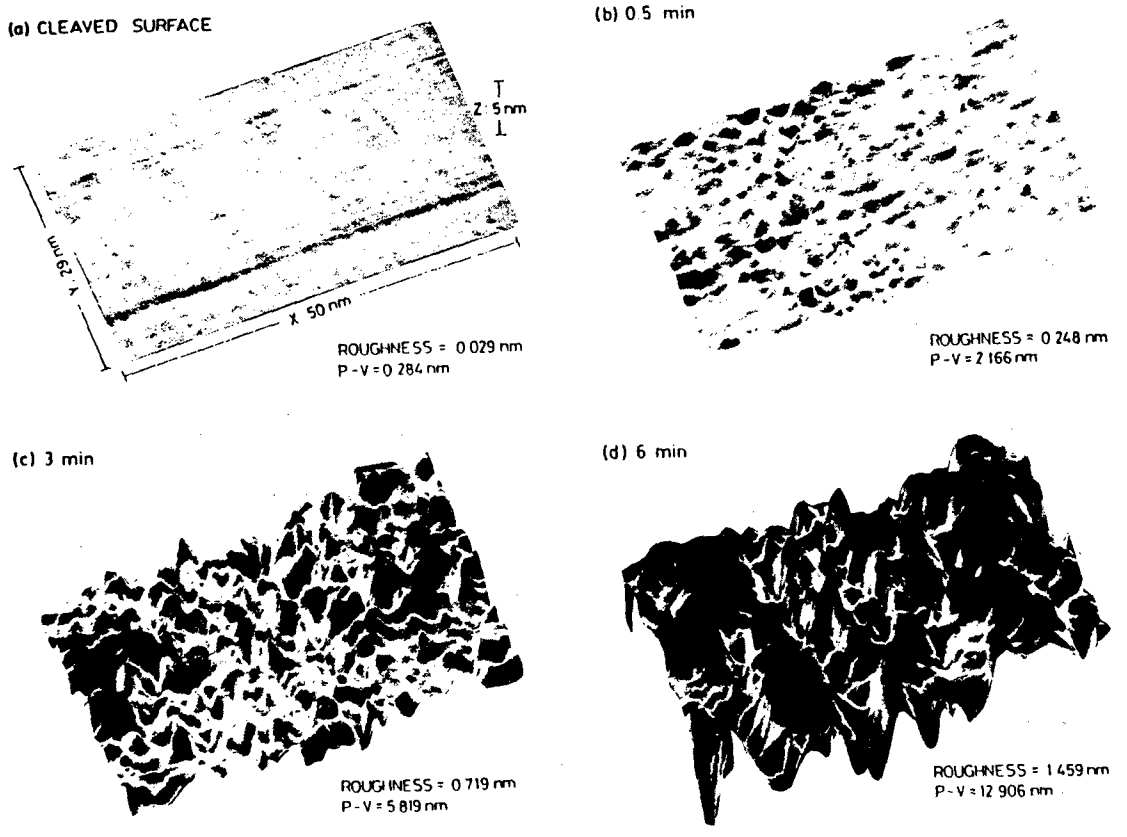


Fig 1 STM imgages of etched surfaces of Kish-Graphite : (a) cleaved surfaces, no etching, (b) 0.5 min, (c) 3 min, (d) 6 min

(2) X ray microscopy

The resolution of optical measurement methods is limited by the wavelength of the light beam. To improve on this resolution limit, it seems natural to use shorter wavelength light, i. e., X-rays. X-rays of 1 nm - 4 nm wavelength are absorbed selectively by materials forming biological specimens. Therefore we can get high resolution images by X-ray microscopes. Walter type reflection mirrors or zone plates are usually used to focus X-rays. We have chosen to focus on fresnel zone plates and phase zone plate in our study of X-ray microscopy.

(3) Study of the optical constants of different materials

It is important to get accurate information about the optical properties of materials which we will use to make multilayer mirrors

and zone plates for use with soft X-rays. We estimate the optical constants by analyzing reflection curves for various wavelengths taken by the reflectometer at the SOR (Synchrotron orbital radiation) facility of Tsukuba high Energy Physics Research Center. Materials are Silicon, Titanium, Nickel and so on.

2.2 Measurement and Control Group

(1) Nanometer position control system

In precision control system, important elements include the feedback system, structure of the mechanism, actuator, and position sensor among other things.

A one-axis stage mechanism for 1-nanometer positioning has been developed using an actuator, newly designed AC synchronous linear motor and and rolling ball guide and yielding a resolution of 1 nm and a maximam speed of 200 mm/s. And it becomes clear that the balls of the guide have special property like spring suite for nanometer positioning. These are shown in fig 2 and 3.

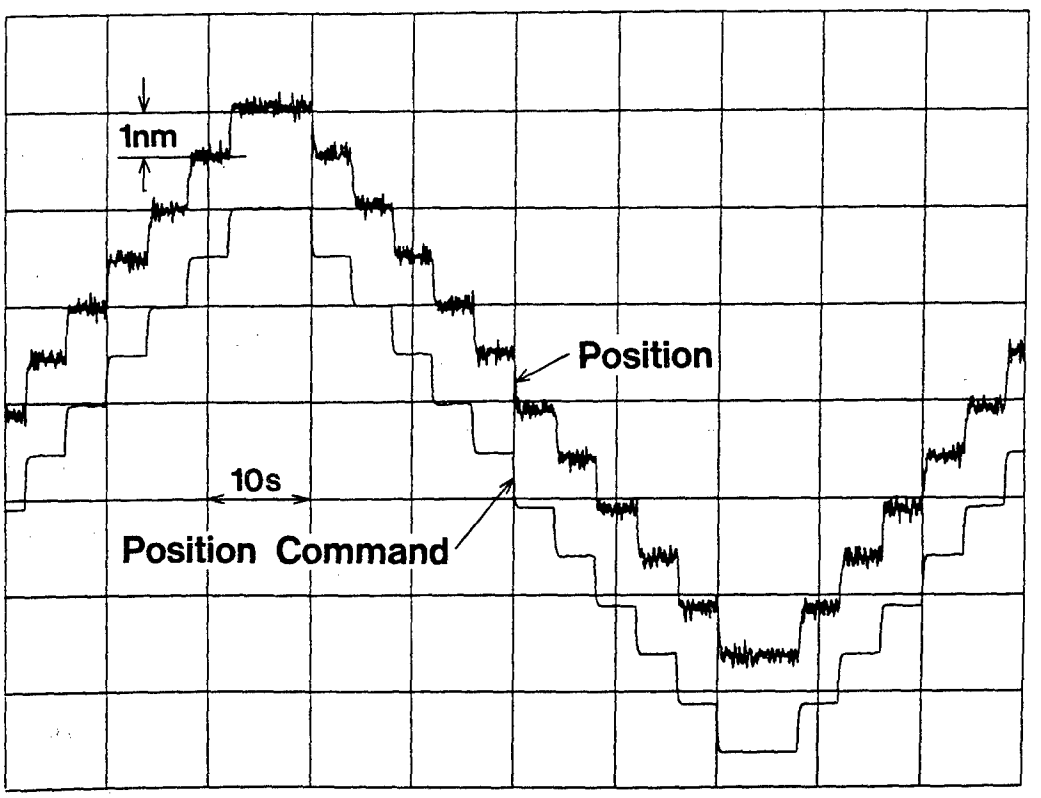


Fig2 1nm step Responce

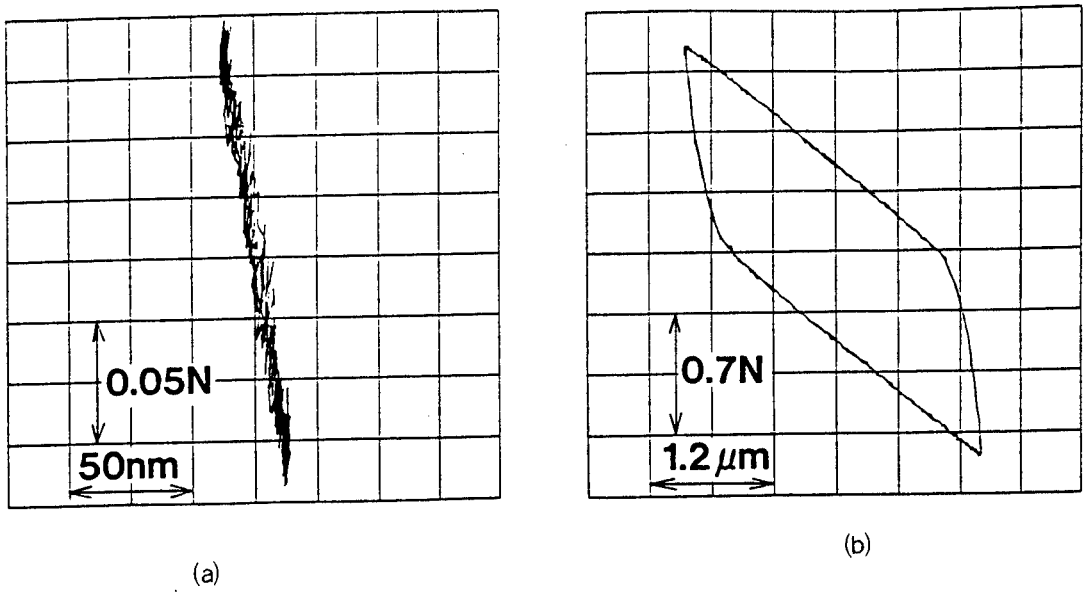
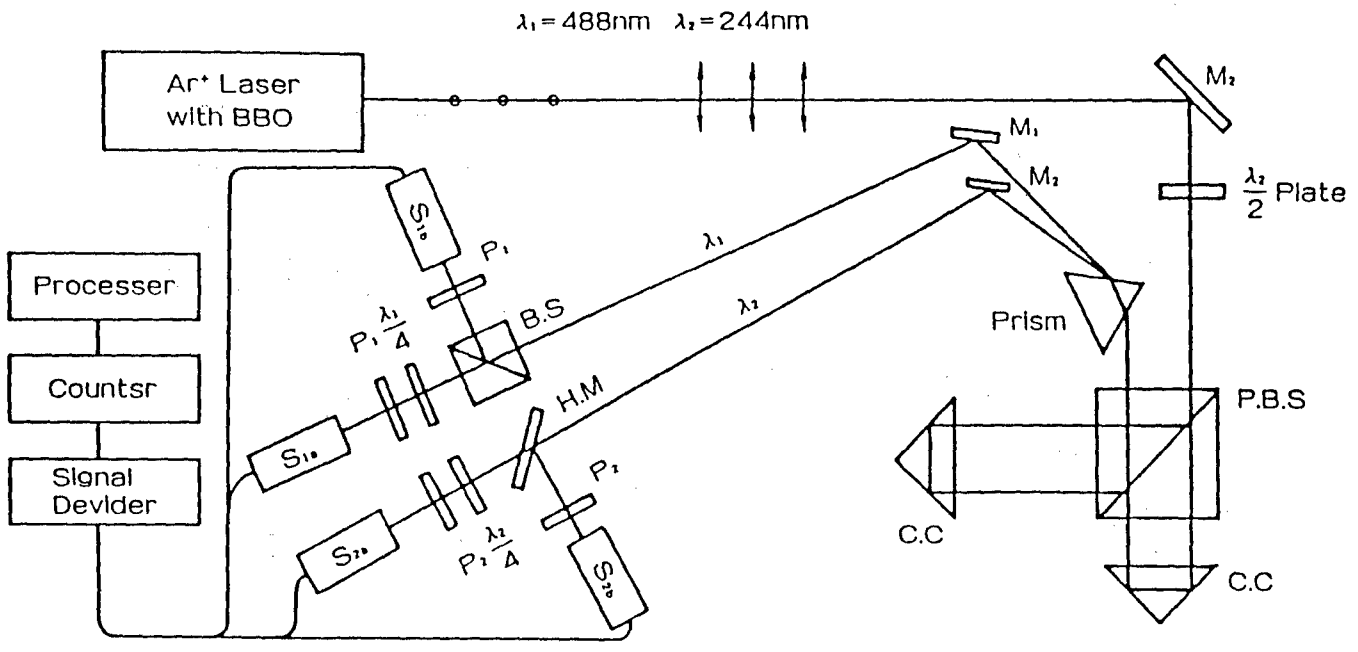


Fig3 Balls Behavior in Nanometer Resion

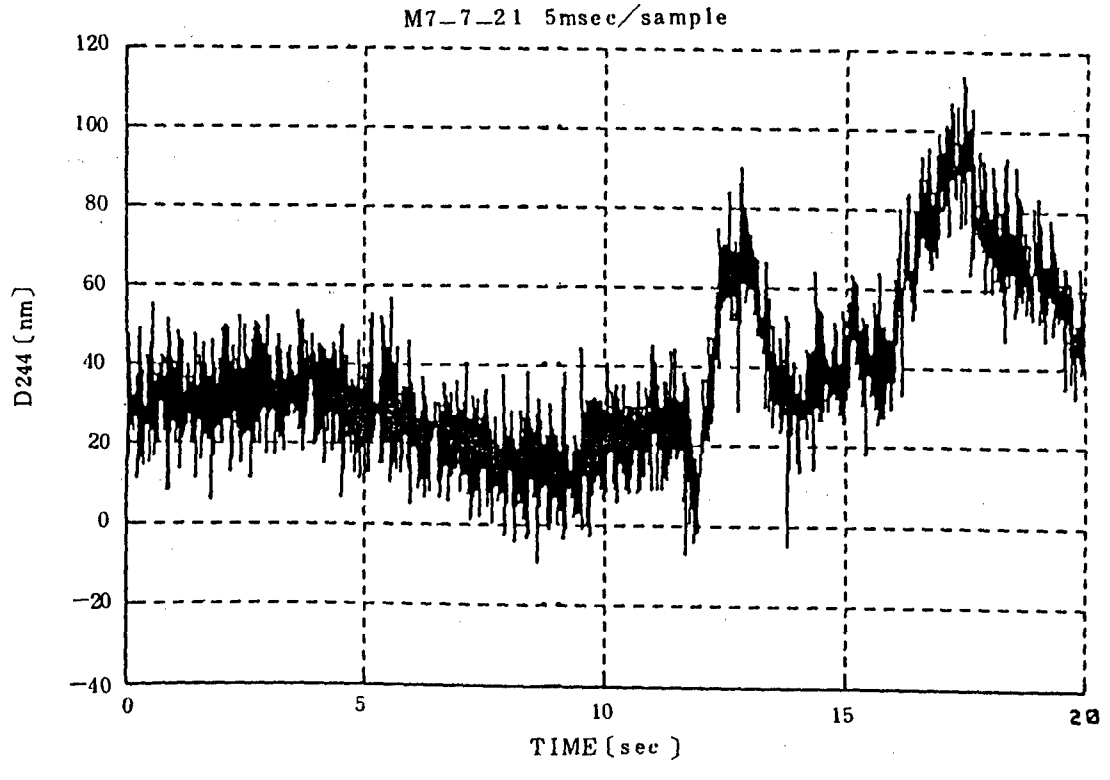
(2) Nanometer measurement system

He-Ne laser interferometry linear displacement measurement systems are already commercially available and being widely used. Resolutions as good as 1 nm have already been achieved in some of these systems. However, these systems are very sensitive to their environment, especially air turbulence. We are studying a two wavelength laser beam interferometer which eliminates errors caused by air turbulence. A 488 nm Ar laser beam and a 244 nm laser beam, which is made by SHG (BBO: ß-BaB_2O_4) from the 488 nm beam, go through same optical path in specially designed optics. The corrected displacement lengths D is given by $D = D_1 - (D_1-D_2)A$, where D_1D_2 are the displacement measured by the two laser beams $\lambda 1$, $\lambda 2$, and A is a constant given by the two wavelengths and the characteristics of air dispersion. Fig. 4 shows optical system of this interferometer, and Fig. 5 shows the measured datas.



M : mirror
P : polarizer
S : detector
C.C : corner cube
P.B.S : polarized beam splitter
H.M. : half mirror

F:g 4 Schematic diagram of a two-wavelength displacement-measuring interferometer



(a) λ2 measurement (original)

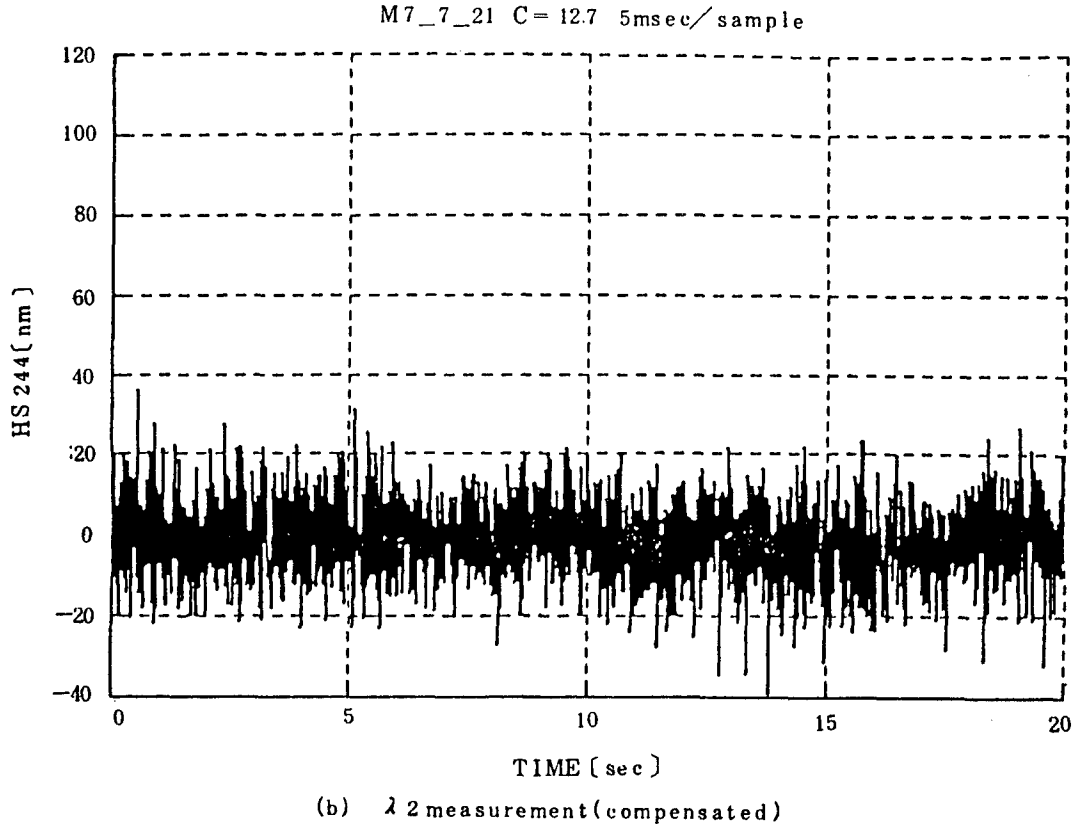


Fig5 Elimination of air turbulence

(3) Small distance positioning system

For the aplication to the wide range scanner of STM or to the alignment system of such like lithography equipment of semiconductor manufacturing, small distance positioning system with subnanometer resolution and several millimeter stroke is very important. We are studying new device for this application.

(4) Micromechanism.

For handling a small size object, small size machine is suitable. We are studying micro linear motor made of silicon fablicated by semiconductor fabrication technology.

2.3 Processing Group

(1) Fabricating X-ray multilayer mirrors

A multilayer coating is needed to reflect X-rays in normal

incidence optical systems, from which we will make X-ray microscopes, X-ray lithographic steppers and other scientific instruments. The reflectivities of multilayers made from a wide range of materials have been calculated using the Fresnel equation for 1 to 5 nm wavelengths. We have discovered that the Ni/V combination should give a high reflectivity. We are now studying how to make such a combination in addition to the popular W/C combination.

The production of uniform, large-size, thin multilayer films by sputter deposition for X-ray mirrors is being pursued. Work is also being done on using photo-CVD methods for the production of multilayer films with an emphasis on improving their densities through good control and uniform deposition.

The TEM Micrograph of W/C multilayer is shown in Fig 6.

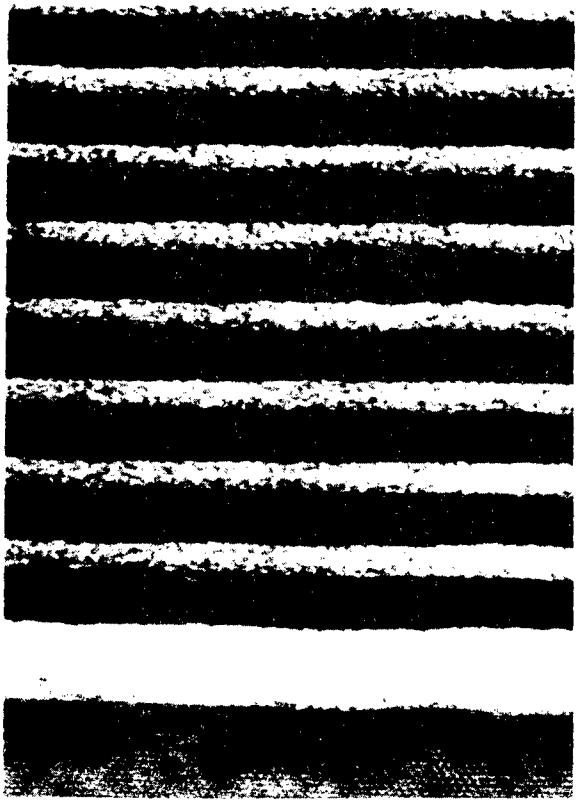


Fig6 TEM Micrograph of W/C Multilayer
d = 3 nm , N = 30

(2) Beam processing to make ultra smooth surfaces

We are using low-energy ion/atom beam sputter etching to try to produce atomically smooth surfaces. Such surfaces are needed as substrates for X-ray multilayer mirrors. Reactive ions being used instead of noble gas ions so that the etching uses both physical and chemical reactions. Low-energy processing is expected to cause less damage to the processed surface.

(3) Study of new methods for reflecting X-rays

The study is also being carried for new method and materials for reflecting X-rays. One idea is use of intercurration. We can immure some kind of metalic atoms in the stages of graphite cristal. Maybe that will be act like multilayer.

X-Ray Microscopy

Hisao Fujisaki and Nobuyuki Nakagiri

Basic Analysis Group

How is it possible to observe the structure and intracellular movement of living cells with high resolution? Light microscopes can be used for specimens in water, but have only limited resolution (approx. 0.3 μm). This is due to the long wavelength of visible light. Electron microscopes have good resolution (order of Å) but cannot be used for wet specimens. This is because the method operates in a vacuum. X-ray microscopes have advantages over both of these microscopes. X-ray microscopes can be used for wet specimens with moderately good resolution. Absorption of 2.4-4.3 nm X-rays by water (composed of H and O) is much lower than the absorption by proteins (mainly composed of C, H, O, and N). The difference in absorption enables us to get images of the inside structure of living cells in water with good contrast.

Reflection mirrors (Wolter type) or zone plates are usually used to focus X-rays. High-angle X-ray mirrors should have shorter focal lengths and would be more convenient to use than low-angle ones. Such mirrors are under development by the Processing Group of our project. The refractive index of materials for X-rays is too close to unity to make X-ray focusing lenses. Zone plates utilize the diffraction of X-rays to focus them. Ordinary zone plates are constructed by alternating transparent and opaque rings. The focusing efficiency is only 10%. This low efficiency requires strong X-ray sources and/or long exposure times. This problem might be partially solved by the phase zone plate.

The phase zone plate replaces the opaque zones of the ordinary zone

plate by transparent ones. This shifts the phase of the X-ray by π-radians during the X-rays passage through the zones. The transmitted amplitude is doubled and the focussed X-ray intensity is 4 times normal. Since the transmitted signal is usually attenuated, the key point for the achievement of phase zone plates is to find materials which have good transparency at the desired thickness to induce a π-radian phase shift.

Twenty-four elements were examined as possible phase zone plate materials for soft X-rays between 0.6 and 11.4 nm, by calculations using optical data. Elements of the fourth row of the periodic table were found to be good for the 2.4 to 4.3 nm wavelength region. These wavelengths are suitable for observing biological specimens. Elements of the fifth row are good for longer wavelengths (6-11 nm). For example, Ag is especially good in the vicinity of 9 nm. This wavelength region is suitable to observe the phosphorus which makes up bones, teeth, DNA and so on.

We have designed a new type of zone plate, the gradient refractive index phase zone plate (GRIPZP) for soft X-rays as part of an effort to make the zone plate X-ray focusing efficiency as high as possible. The GRIPZP is based on radial modulation of the refractive indices of the materials. It is made with two materials: the concentration of one material increases gradually and that of the other decreases with increasing radius in each pair of zones. When such a zone plate is made with titanium and chromium and their concentrations are optimized for use with X-rays of 2.74 nm wavelength, the focusing efficiency should be 34%. This value is 3.4 and 1.4 times the efficiencies of the Fresnel zone plate and the π-radian phase shifting zone plate, respectively.

The X-ray focusing efficiency of the phase zone plate is now under experimental investigation. We use a phase zone plate made of nickel which was designed for use with X-rays of 1.76 nm wavelength. We also

use a Fresnel zone plate made of gold for comparison. X-rays are generated by an iron thin foil target with a focused electron beam. The images of focused X-rays are taken by a commercially available automatic camera in a vacuum or a microchannel plate with a fluorescent screen and a TV camera. In the latter case, the image data can be recorded on video tapes, and processed by a computer.

Nanometer Positioning Micro Dynamics

Akihiro Furutani

Shigeru Futami

Measurement and Control Group

In our previous experiments, a 200mm/s maximum velocity and 1nm step response were achieved by using a one-axis stage mechanism having a 250mm stroke, driven by an AC linear motor and guided by an rolling ball guide mechanism.

Because position control in the nanometer region heavily depend on the characteristics of the mechanism, we examined the micro dynamics of the stage. In our apparatus, the motor generates the driving force without contact and the moving stage is supported by the balls of the guide mechanism only.

The measurement of differential force relationship by closed loop position control gave a graph of the relationship consisting of the following three regions.

Region I: Less than 100nm difference. The force is completely proportional to the difference, so the dynamics of the guide and the stage can be modeled as a spring oscillator with one degree of freedom. The spring constant is almost 7N/μm. The natural frequency and the damping ratio are about 200Hz and 0.05.

Region II: From 400nm to 100 m difference. Hysteresis occurs in this region. The relationship between the two variables is approximated by a line when the difference is fairly small. The inclination of the line is less than one-thirtieth of that in Region I.

Region III: More than 100μm difference. The force is saturated in this region and the dynamics of the stage can be treated as a rigid mass acting on by normal rolling friction. Almost all movement in a long stroke positioning is carried out within this region.

The open-loop measurement of differential force relationship showed that constant rolling does not occur in region I but does in regions II, III. The stage returns to the equilibrium position very quickly in region I when the force is released. Forced oscillation measurement indicated that the dynamic model in region I is correct for high frequency responses of more than 200Hz. The damping ratio of the system is calculated from the peak of the gain diagram from this measurement.

The force-velocity measurement from the closed-loop responses showed that at the start of movement in this system, the force increases monotonously against the velocity and there is no static friction force larger than the constant rolling friction force.

Particle Beam Processing for Producing Super Smooth Surfaces

Makoto Honda

Processing Group

Creates precision and super smooth surfaces are required for the development of soft X-ray multilayer mirrors and high power lasers.

The most precise machining methods, elastic emission machining and float polishing can achieve damage free and super smooth surfaces which have subnanometer rms roughness.

Using a new approach, we investigated the possibilities of producing clean super smooth surfaces by a dry process instead of the wet process using slurry. Here, ion beam sputter etching was carried out and the surface roughness change investigated.

Two methods were used to measure the surface roughness. The first was optical profiling using phase-shift interferometry (WYKO Corp., TOPO3D), and the second was the X-ray scattering method based on the first order vector perturbation theory. The difference in the range of spacial wavelengths measurable must be considered. The spacial wavelengths measured by the former method is relatively greates in the range 4 - 500μm, and the latter method measures in the range 1 - 12μm.

The continuously rotated tungsten thin film surfaces were etched by 0.25keV and 1.1keV Ar ion beams under the conditions of incident beam angle 0 - 85° (0°; perpendicular direction).

No changes in the roughness in the 4 - 500μm range were detected by the optical profiler, but changes in the 1 - 12μm range were measured by X-ray scattering. The changes were smaller when using the lower energy level and higher incident angles. Especially, with the 0.25keV beam and incident angles higher than 70° it is clear that the surfaces could be etched with no changes in the roughness in the 1 - 500μm range.

Masuhara Microphotoconversion Project

New Chemistry by Laser and Microfabrication Techniques

Hiroshi Masuhara

Project Director

Microphotoconversion is concerned with a new system in which molecules and materials are converted by controlling chemical reactions in extremely small volume of the order of micrometer with picosecond time resolution.

Chemical reactions, if looked at over short time periods, consist of several elementary processes in combination. Also, chemical reactions proceed generally in inhomogeneous field. The fundamental understanding of chemical reactions and the construction of the efficient conversion system of molecules and materials will be accelerated by elucidating structural and reaction dynamics in the micrometer-size reaction volume. This idea is supported by the fact that biological organisms, an ultimate form of chemical reaction system, are a highly advanced conversion system which performs its function by suitably combining and controlling transient processes in spatially arranged minute reaction fields.

Lasers provide monochromatic, intense, and short-pulsed light that can be focused to a very small spot. This has been used as a tool to clarify elementary processes such as excitation energy relaxation as well as both electron and proton transfer in the nanosecond and picosecond time regions. Also, chemical reaction mechanisms have been elucidated for isomerization, dissociation, cyclization, and oxidation-reduction reactions by means of time-resolved laser spectroscopy.

Laser light has also been an energy source for chemical reactions

and material processing. Furthermore, it is recognized that the laser offers potential means to adjust local environmental reaction conditions such as polarity, viscosity, pH and reactant concentration. Molecular conformation and sol-gel transition of polymers can also be controlled by light.

On the other hand, microlithography, laser ablation, and scanning tunneling microscope have greatly advanced in recent years. It is now possible to prepare micrometer-size spots and to study their chemical function. Consequently, a conversion system where such minute reaction sites are arranged spatially will probably be constructed. Laser light with micrometer resolution should be utilized in order to energize, interrogate and control chemical reactions in this system. Thus, we believe that new chemistry will be developed by combining laser and microfabrication techniques in molecular and materials systems.

In this project the research is started by studying dynamic structure and chemical reactions in micrometer-size volume by means of picosecond laser spectroscopy. On the basis of these results, we explore a new molecular and materials conversion system where temporal and spatial arrangements of the minute reaction sites are given. This will contribute to various kinds of technology related to lasers and materials in addition to chemistry.

The project consists of three groups dealing mainly with lasers, microfabrication techniques, and reaction design. In the past one year we have recruited researchers, prepared the office and laboratories, and set facilities. Now eleven researchers including a foreign chemist are working, and four researchers will join in the coming months.

(1) Dynamic Microspectroscopy Group

(in Kyoto Research Park Co. Ltd., Kyoto)

The time-resolution of laser spectroscopy which can be applied to various kinds of molecular assemblies and materials is improved up to picosecond, and micrometer-order spatial resolution is also provided. This is a new class of methods elucidating dynamics and reactions in minute functional sites.

A time-correlated single photon counting system, using a synchronously pumped, cavity-dumped dye laser, excited by a CW mode-locked Nd^{3+}: YLF laser, as an excitation light source is constructed. The full-width at half maximum of instrumental response function is 30 picosecond. Precise and accurate measurements of time-resolved spectra and fluorescence rise as well as decay curves are possible in the picosecond domains.
A fluorescence microscope with quartz lenses was installed, and a confocal laser scanning fluorescence microscope was set up. A depth information with the resolution of a wavelength is obtained in addition to two-dimensional resolution. This is combined with the single photon counting system, which gives molecular and electronic information with micrometer and picosecond resolutions (Fig. 1).

Surface and interface problems also require an analytical method with high spatial and temporal resolutions, which has been realized by introducing total internal reflection condition. Namely, fluorescence and visible-ultraviolet absorption from the surface and interface layers with the thickness of submicrometer are now measured in the picosecond time region.

Such temporal and spatial analysis of dynamic structure and chemical reaction can be made by developing a new data processing. This has been already achieved by introducing a convolved autoregressive model fitting.

(2) Microchemical Function Group

(in Central Research Laboratories, Idemitsu Co. Ltd., Chiba)

Using fabrication techniques, micrometer-size spot with chemical function is created on the surface of polymers, metals, and semiconductors. Conventional microlithography and laser ablation are fruitful in patterning various kinds of materials, and we are also introducing a scanning tunneling microscope for this purpose. These facilities are set in a class 100 clean room.

We have started some studies on fluorescence dynamics of adsorbed molecules on gold microelectrode patterns which were fabricated by a conventional microlithographic method. Fluorescence from the finger printed electrode shows different behavior from that on the glass substrate (Fig. 2).

Fabrication of polymer films by laser ablation is also performed. Not only a spatial patterning but also a characteristic photochemical reaction due to laser multiphoton absorpotion are possible and now being explored.

(3) Microconversion System Group

(in Research Institute for Production Development, Kyoto)

This group explores a new integrated conversion system in which reaction steps in micrometer-size spot are driven, controlled, and interrogated with laser light and which will be highly efficient and selective in molecular and materials conversion.

In this year, some approaches have been conducted for planting photo-functional molecules onto the surface of various kinds of materials. We have succeeded to provide photo-functionality by introducing photochemically an isomerizable molecule on polymer surface. This means that a surface patterning by photo-functional molecules is attained (Fig. 3).

Decreasing the size of chemical reaction field and examining its effect are another important subject in this group. We prepared fine particles and thin films of photoresponsive gel and compared their photo-induced sol-gel transition to that of bulk gel. One of the interesting results is that the response of microparticle is faster than that of the bulk one. We consider this type of size effect is crucial and elucidate it by utilizing our time-and space-resolved laser spectroscopy.

Nishizawa Terahertz Project

- Exploration for Terahertz Semiconductor Devices -

Junichi Nishizawa

Project Director

The purpose of this project is to research the semiconductor devices and circuits which operates in the Terahertz (THz: 10^{12} Hz) region. Terahertz region is the intermediate band between the light wave and the radio frequency.

The semiconductor devices are used up to 100GHz (10^{11} Hz) and optoelectronic semiconductor devices such as the semiconductor injection laser are the key device in the optical communication systems.

The Terahertz semiconductor devices and their related circuits will expand the usable frequency region from submillimeter to infrared.

There are three subgroups in our project located in Sendai; Basic Analysis, Functional Device and Circuit Configuration. In the Basic Analysis Group, the photo-stimulated molecular layer epitaxy are used to obtain the very thin epitaxial layer for the very high speed transistor and diode. Several kind of devices such as the ideal static induction transistor, Tunnett diode, semiconductor Raman laser etc are developed now. The circuits which operate as the mixer, detector, harmonic generator, travelling wave type optical modulator etc are also investigated.

Here we report in more detail of our project.

Molecular Layer Deposition of Metal

- Aluminum deposition with organic aluminum compounds -

Noriaki Manada

Basic Analysis Group

In Prof. Nishizawa's perfect crystal projects, photo-exited molecular layer epitaxy was established. This method was able to fabricate single crystals of GaAs, $Al_xGa_{1-x}As$ and Si monolayer by monolayer.

In this study, we tried to fabricate the film of metal by applying the molecular layer epitaxy. As aluminum source, dimethyl aluminum hydride was used, the deposition was performed at the temperature below 250°C. The specific resistivity of this aluminum deposition was achieved as about three times as that of the bulk aluminum.

So, we judge that dimethyl aluminum hydride is a good source producing the electrode of devices. Schottky diode and ohmic test devices were formed and the electric charactaristics were measured. However, the surface morphology of the deposited film of aluminum is not good, it is necessary to improve more mirror surface condition.

Heterojunction Semiconductor Raman Laser

Ken Suto

Functional Device Groupe

Semiconductor Raman Laser is being developed for use in a very wide band optical communication in which the band width exceeds 1 THz. It can be used for an optical heterodyne demodulation of a wide band optical signal, as well as optical mixing and optical amplification. We have realized buried heterostructure semiconductor Raman laser by a system of GaP and $Al_xGa_{1-x}P$, and attained the threshold optical input power less than 1W. It is possible to realize a practical semiconductor Raman laser, which is pumped by a laser diode, by further improvements of the structure and crystal perfectness.

Terahertz-Band Circuits and Measurements

Koji Mizuno, Yuitsu Hayashikura

Circuit Configuration Groupe

The THz region of the electromagnetic wave spectrum lies between the microwave and visible regions. Both the microwave and visible regions are fully utilized, but in order to use the entire spectrum for the benefit of mankind, we must develop the THz region. The Circuit Configuration group is now studying circuits for devices and measurement instrumentations for the development of THz region technologies.

A quasi-optical cavity oscillator is being developed using an array of many active devices. We are studying this structure by using model experiment at 10GHz-band. We have observed oscillation of Gun diodes array of 5x3 and FETs array of 3x2.

We have been studying quasi-optically coupled harmonic mixer which is very useful for frequency measurements and spectrum analysis at the THz region. This mixer has quasi-optical structure for a THz signal and waveguide structure for local oscillator mm-wave power. A Schottky diode was used for this mixer because of its strong nonlinearity. A CH_3OH laser power of 2.5THz was converted to intermediate frequency of 1GHz by mixing with the 25th harmonic of 100GHz local oscillator power.

At the THz region, a Schottky diode has been used for detectors and mixers with a combination of 4-λ antenna with a corner reflector. We have calculated voltage sensitivity of the detector in order to optimize the diode structure. This analysis indicates that smaller capacitance, that is, smaller diameter, is important to increase the sensitivity. For detector/mixers, not only sensitivity but also noise chracteristics of diode's performance is important. In fabrication processes of diodes, dry etching process and further surface treatment increase defects at GaAs surface. Correlation between trap density at the interface of metal/GaAs and noise characteristics has been calculated theoretically. This analysis can explain the experimental results of noise measurements.

Research of High Frequency Optical Waveguide Modulators

Makoto Minakata

Circuit Configuration Groupe

Optical waveguide modulators are important devices for light transmission, switching and signal processing systems. Efficient and broad-band optical waveguide modulators using $LiNbO_3$ and GaAs have been reported. For a traveling-wave optical waveguide modulator, the maximum modulation bandwidth is limited by the difference between the optical wave and modulating wave phase velocities.

In this work, a new structure of travering wave optical modulator which offers a potential of perfect velocity-matching is proposed, and characteristics and design of the modulator have been analized and calculated exactly. The modulator comprises of a $LiNbO_3$ ridge waveguide, a loaded strip layer with a low dielectric constant and vertical traveling-wave type electrodes. Preliminary experimental results show that the modulating wave velocity is speed up without decreasing the applied electric field, and the experimental values agree with the theoretical ones.

Quantum Magneto Flux Logic Project

- Supercomputers through Superconductors -

Eiichi Goto

Project Director

The advancement of supercomputers has made it possible to analyze many complicated natural phenomena, such as meteorology, ULSI's and even DNA's. However, more than several tens of times faster computers are required to accurately analyze these large systems. Silicon ULSI should face limitations arising from heat consumptions and signal delay in such fast operating systems. Therefore, Quantum Flux Parametron (QFP) is a possible candidate for the future supercomputers because of its low power consumption (10^{-9}W) and fast switching speed (10^{-12}s) with its capability for three dimensional packaging through magnetic coupling. This project aims to demonstrate (1) the fundamental GHz operation of QFP device/circuit (2) the capability of removing magnetic flux from the superconductors and (3) operating system suitable for QFP computer.

Quantum Flux Parametron as a high-speed logic device and its 3-dimensional packaging

Ryotaro Kamikawai

Fundamental Property Group

Quantum Flux Parametron (QFP) is expected to achieve high-speed operation with low power dissipation. Moreover, since the information is carried by magnetic flux, it is possible to send signal through a transformer, which enables non-contact connection between circuit modules. These characteristics are in favor of high-density packaging, and thus QFP is expected to take the place of high-speed Si devices for computers in the 21-st century.

DC functional operation of a three input majority circuit has been successfully observed. In addition, it is expected that logic functions used frequently in computers such as a multiplexor and a parity generator are efficiently constructed using QFP's.

High-speed operation at 5GHz has been demonstrated by fabricating a 1/2 frequency divider. Since the frequency is limited by the package rather than the circuit itself, experimental efforts are being continued for demonstration at higher frequency.

A feasibility model is being designed to demonstrate inter-chip, non-contact signal transmission through transformers. An inductance calculation program for 3-dimensional conductors has been developed and used in the physical design of the model.

Implementation of a Cyclic Pipeline Computer

Norihiro Fukazawa

Computer Architecture Group

A new computer architecture, called Cyclic Pipeline Architecture (CPA), is being studied as a suitable architecture for computers which consist of Quantum Flux Parametron (QFP) devices.

The CPA is based on pipelining which is an implementation technique to provide a better cost/performance and requires latching between each pipeline stage. Since the QFP logic device acts as a latch, it is well suited for an architecture like the CPA. The CPA implements a MIMD (Multiple Instruction stream Multiple Data stream) computer by timesharing the processor and the main memory among the multiple instruction streams, thus creating virtual processors. By sharing pipeline stages among several virtual processors, pipeline dependencies which degrade the performance are minimized, resulting in an increased performance.

Currently, a Cyclic Pipeline Computer (CPC), called FLATS2, is being implemented using silicon technology. The FLATS2 is designed to give high performance in numerical and symbolic computation. During the implementation of FLATS2, a special architectural feature called BL addressing is proposed and implemented. In BL addressing, address calculation and the range checking is done in parallel, by specifying both the range (Base and Limit) and the address for each memory access. It pvodies a way to improve the performance in array accessing, which is one of the most important optimaizations for numerical application. It also provides several efficient functions for language systems, such as FORTRAN, LISP, C language, which are now being implemented.

An operating system for CPC called CPX is now under development. In developing the CPX, the kernel which only has the minimum functions is constructed first, and then, the other functionalities of CPX are to be implemented as user programs on top of the kernel. The implementation of the upper level functionality of the operating system as a user program allows us to experiment with various versions and evaluate the effectiveness of the CPC. The CPX kernel is a message passing, object oriented kernel for CPX operating system. It aims for the efficient usage of the shared memory multiprocessor structure of the CPC. The basic functionalities of the CPX kernel are confirmed on the FLATS2 instruction simulator, through the implementation of XINU operating system on top of the CPX kernel.

Perfect Magnetic Shielding and a Novel 4K Refrigerator

Junpei Yuyama

Magnetic Shield Group

One of the purposes of our research is to achieve a low magnetic field so as to guarantee a normal operation of the Quantum Flux Parametron (QFP). Although the superconducting magnetic shield is the only method to achieve such a low magnetic field, it is known that this method involves a serious problem. The problem is that some flux lines are trapped within the superconducting vessel wall while the vessel is being cooled down through the transition temperature. Since these flux lines produce a magnetic field inside the shielding vessel, we have not yet obtained any null-magnetic-field space.

We have proposed a method to sweep the trapped flux lines away from the vessel wall by using a hot normal spot. This method is referred to as Micro Heat Flushing (μHF). In order to investigate the potentials of μHF, we must establish a technique to detect flux lines trapped within the superconductor. The basic concepts of our detection technique are as follows:

(1) Keep the gap distance constant between the superconductor surface and the pick-up head by allowing helium gas to blow out from the center of the head and flow radially through the gap between them.

(2) Mount a pick-up coil on the head, and connect the coil to the SQUID magnetometer.

(3) Measure the magnetic field distribution just above the superconductor surface by scanning the pick-up coil over the surface while keeping the gap distance constant.

(4) Deduce the distribution of trapped flux lines from the magnetic field distribution.

For the first step, a room-temperature experiment on the gap control by gas flow is conducted. The pick-up head can be firmly suspended by a radial gas flow, and the gap distance is well controlled by adjusting the supplying pressure. We are proposing a gas-suspension model based on the boundary-layer theory. Our model well explains the room-temperature experiment, and suggests that the head be suspended with a 15-25μm gap at a liquid helium temperature. Low temperature experiments on gas suspension and flux detection are now under way.

The other purpose of our research is to realize an efficient and long-life 4K refrigerator. We are now developing a long-life bellows to be used at cryogenic temperatures in order to construct a bellows expansion engine. Our idea is to make multilayer bellows by means of layer-by-layer deposition of soft and hard metals. Stress analysis is made by the finite element method (FEM). This analysis shows how the maximum stress in multi-layered bellows depends on the working pressure and the stroke. Nickel-lead multi-layered bellows are made by an electrodeposition technique. Fatigue tests are currently in progress.

Furusawa MorphoGene Project

- Searching for Gene which Controls Development -

Mitsuro Furusawa

Project Director

1. Overview

The phenomenon that a fertilized egg forms a complex and delicate organism through repeated divisions and differentiation into various types of cells is one of the most attractive themes in developmental biology.

"MorphoGene" is a general term describing the genes which control morphogenesis in the process of ontogeny. Detailed studies of the relationship between morphogenesis and tissue interactions have already been made by transplanting embryonic tissues using amphibians, chickens, sea urchins and other species. Simultaneously, molecular biologists have been trying to understand living organisms at the molecular level by investigating how DNA (genes) determines the structure of proteins and how gene expression is regulated. Genetic analyses of mutants in *Drosophila* and *Caenorhabditis elegans* (a nematode) have revealed the causal relationship between gene and phenotype at the molecular level.

Recently, a DNA sequence called a homeobox has been identified in homeotic genes which determine the direction of differentiation of each segment in *Drosophila* larvae. Since this homeobox DNA sequence is common to many animal species, including human, the possibility can be realized for understanding morphogenesis at the molecular level.

The main themes of this project are to search for the genes that control the fundamental processes of gastrulation and differentiation of germ cells (sperm and ova). To attain these goals, we are developing new methods for recognizing subtle differences in DNA and RNA structure in various cell types which represent different stages of development. By starting from the DNA (genes) or RNA (cDNA or mRNA) cloned with these new

methods, the specific genes involved in development can be inferred by determining the function of their gene products (proteins) using monoclonal antibody approaches. Thus, we will finally be able to clarify the roles of these genes in development. Also, new methods for producing transgenic mice will be developed for the purpose of investigating the regulatory mechanisms for specific gene expression. These animals should serve not only as tools for searching "MorphoGene", but also as good models for human genetic diseases.

The project is being carried out by three groups: the Gene Search Group, the Gene Expression Group and the Gene Control Group. The following briefly summarizes the current research activities of these groups:

1) Gene Search Group

It is known that the eggs from *Xenopus* female frog, named No. 65, stop developing just prior to gastrulation and lack a 38K protein which is present in wild-type eggs. We are cloning the specific cDNA that codes for this protein from the wild-type eggs using the (λ)gt 11 immunoscreening system and have obtained several positive candidates. In addition, we are pursuing mRNA colnes that facilitate the onset of gastrulation in *Xenopus*.

Concerning the study on germ cell differentiation, we are initiating studies to make specific monoclonal antibodies against primoridal germ cells in tadpoles. These antibodies will serve as markers for germ cell differentiation.

2) Gene Exspression Group

We are testing several alternative model systems to improve the sensitivity of techniques which identify differences in the mRNA or DNA between two cells or tissues.

3) Gene Control Group

We are constructing specially-designed DNA sequences that include antisense β-globin genes. These DNA sequences will be introduced into mouse eggs for the purpose of producing transgenic mice with anemia. These studies will provide insight into the regulatory mechanims of gene expression. Moreover, several trials are in progress using the transgenic mouse system to attain the site-directed integration of foreign DNA into the mouse chromosomal DNA.

2. Gene regulation by steroid hormones

Minoru S.H. Ko

Search for Genes Group

Steroid hormones, a group of cholesterol-derived lipids, are known to act as a direct regulator of gene expression. In brief, the hormone first binds to its receptor protein to form an active receptor-hormone complex, which bind to a specific DNA sequence called Hormone Response Element (HRE) located at the promoter region. Interaction between the receptor-hormone complex as well as other regulatory factors and HRE results in transcriptional activation of such steroid-inducible genes. Selective modulation of gene expression by steroids can thus be considered as an excellent system to study eukaryotic gene transcription in general. Here we show two experimental systems; one represents a novel, highly inducible gene expression system mediated by glucocorticoid, a member of the steroid hormones, the other gives insight into the basic mechanism of the steroid-inducible gene expression.

(1) A highly inducible system of gene expression

A new gene expression system in mammalian cells was developed by using the glucocorticoid receptor (GR) as an inducible positive feedback factor. Mouse Ltk$^-$ cells were transfected with plasmids carrying the GR-encoding gene and the β-galactosidase reporter gene, both of which were fused with the glucocorticoid-inducible promoter of the mouse mammary tumor virus (MTV). The GR gene was first induced to supply the receptor protein, which further induced the expression of both GR and reporter genes. This system allowed us to increase the expression of the reporter or target genes without augmenting basal levels of expression significantly, and may be useful to investigate the unknown function of a cloned gene, particularly when the gene product of interest is cytotoxic or growth-inhibiting.

(2) Heterogeneous and stochastic nature of glucocorticoid-induced gene expression

Generally, studies of gene transcription have been carried out using in vitro system. As in such a system transcriptional factors and their responsive gene sequences (templates) are in large excess compared to in vivo situation, the results obtained from in vitro studies may not reflect a natural phenomenon. To investigate the activity of an individual template, we isolated a number of Ltk^- cell clones that contain a single or a few copies of β-galactosidase gene under regulation of the MTV promoter. The cells were induced with glucocorticoid for 48 hours and the transcription of β-galactosidase gene in an individual cell was scored histochemically. Unlike the common idea, the level of β-galactosidase gene expression was significantly varied among individual templates, although they were under the same induction condition. Considering the transcriptional frequency of individual templates for 48 hours, our data are interpreted to mean that the utilization of templates for transcription is biased, implying the formation of stable transcription complexes on the MTV promoter and the stochastic switching of the gene expression.

3. Development of a cloning procedure for genomic DNA with an altered primary structure

Hiroshi Yokota

Expression Group

A procedure was developed to clone anonymous restriction fragments with an altered DNA primary structure which distinguishes two DNA samples. The procedure is based upon in gel competitive DNA reassociation after electrophoresis of a mixture of a restriction enzyme-digested target DNA (from which clones are to be isolated) and unclonable reference DNA (competitor DNA).

Using this procedure, we isolated a clone (BL-1) from a rat DNA which gave an apparently amplified DNA band in a specific tissue (brain) when used as a probe for Southern hybridization. The sequence of BL-1 was very similar to a portion of the distal putative open reading frame (ORF) of LINE (Long Interspersed Element) with a possible reverse transcriptase function. Further, we found that poly(A)+ RNA hybridized with BL-1 was enriched in brain. These results suggest that there exists tissue specific amplification of BL-1 containing sequences and the amplification is associated with its transcriptional activation.

Molecular Dynamic Assembly Project

- Flexible Function of Biomolecular Machines -

Hirokazu Hotani

Project Director

1. Overview

Biological organisms effectively perform highly sophisticated functions such as self-organization, self-replication, energy conversion, information propagation and processing, and selective transport of substances. The unit elements which perform these intricate functions are thought to be assemblies of such biological molecules as proteins and lipids.

In the past, the dynamics of molecular assemblies were considered to be deterministic like those of man-made machines, which have unique output responses to given inputs. But as research on molecular assemblies has progressed, various phenomena which can not be explained by this analogy have been discovered. Molecular assemblies have been found to be capable of changing their own energy conversion and information transmission functions in response to environmental changes. Such assemblies are termed "supramolecules".

For example, the flagellar motor, which powers bacterial flagellum rotation, operates as a result of proton flow into the cell through the motor. The relationship between the angle of rotation and the number of protons flowing into the cell is not fixed, but varies according to the environmental condition. Specifically, when the load is reduced, the motor rotates with fewer protons than before; the motor is designed in such a way that it can rotate smoothly even if there are not enough protons flowing into it. Such an input-output relationship is called "loose-coupling" which can also be observed in the acto-myosin system in muscle and Proton ATPase.

2. Research Strategy and Objectives:

The goal of this project is to understand why supramolecules function properly in thermally fluctuating environments and to explore related engineering applications. This could lead to the creation of "intelligent" molecular systems which can change their morphologies and function in response to environmental changes.

Thus, one of the project groups (Fundamental Analysis) is analysing the structures of the flagellar motor, acto-myosin system and flagellar filaments by direct observation. Their microstructures are being analyzed by such means as x-ray diffraction and also, three-dimensional views of supramolecular structures will be obtained by computer graphics, and the functions and principles of the dynamics of supramolecules will be clarified by measuring their momenta and the amounts of such substances as ATP and protons entering and leaving them.

Also, methods for reconstituting supramolecules in vitro with such biological functions as environmental adjustability, self-assembly, self-repair, self-dissociation and memory, are being pursued by creating new three-dimensional structures by modifying the constituent proteins of supramolecules. By utilizing the self-assembly function of superamolecules, attempts will be made to create microstructures whose sizes and shapes can be controlled freely and very precisely while having information transmission and conformation transition fuctions, and whose functions and characteristics can be controlled by varying thermal, light and other inputs.

A search will be made for ways to utilize the characteristics of supramolecules for constructing biosensor and biochip systems capable of sensing, processing and judging external information. Specifically, by utilizing methods of embedding proteins in membranes and of artificially modifying proteins, attempts will be made to endow single supramolecules with multiple functions.

Direct Measurement of Biological Motor Rotation

Seishi Kudo

Higher Functions Systems Group

Bacteria swim in liquid by rotating their flagella, which consist of an external helical filament driven by a rotary motor (flagellar motor) embedded in the cell surface. The flagellar motor is the only known natural molecular rotary machine, and therefore is a true 'biological motor'.

In order to measure directly the rotation of a single flagellum with high temporal resolution, we have developed a new method, 'laser dark-field microscopy'. The system consists of a He-Ne laser, a dark-field microscope, a photomultiplier and an SIT camera.

The following results have been obtained: (1)A single flagellum can rotate at a speed as high as 140Hz at room temperature. (2)The fluctuation of rotation speed was smaller than 10%. (3)Abrupt slowdowns of speed (20-50%) was occasionally observed. Rotation speed recovered from these changes within a few tens of milliseconds. (4)Mutant analysis indicates that the switch complex, a part of the motor, is responsible for stabilizing the speed of rotation.

Molecular process of bacterial flagellar formation

Ichiro Yamashita

Fundamental Property Group

Bacterial flagella are the organelles of motility by means of which bacterial swim in liquid environments. A flagellum is a gently coiled filament composed of a single kind of protein, flagella, and is rotated by a motor at the base of the filament. Flagella adopt a number of distinct helical conformations by perturbations of the environment such as an abrupt change in the direction of rotation that frequently occurs while bacteria swims and changes in pH or ionic strength. Understanding the mechanism of these changes at the molecular level will give us a deep insight into the dynamical properties of protein molecules in general. This requires a structural basis on which the explanations of the observed dynamical behaviors can be obtained.

We have analysed the X-ray fiber diffraction patterns from oriented sols of straight-type flagella at 20Å resolution. The electron desntiy distribution allowed us to resolve the elongated shape of the monomer and its packing. Knowing which domains of the monomer correspond to which parts of the amino acid sequence, we now know the parts of the subunit responsible for its specific functions. The middle part of the primary sequence, which is highly variable and responsible for antigenicity, forms a relatively large domain located outside of the filament and is exposed to the environment. Both terminal regions are conserved and form a relatively small domain at the core of the filament, determining the shape of this assembly independent of the other domain. These regions are partially unfolded and flexilble in monomers, prohibiting monomers from spontaneously polymerizing into filament until they encounter the

specific structure at the distal end of the flagellar filament. Monomers are synthesized in the cell body and somehow transported to the distal end of the flagellum. We have observed a hole of 6nm in diameter at the center of the filament that is large enough to be a channel for flagellin export. Therefore, it is quite likely that flagellin monomers are transported through the central channel to the distal end and added onto the very end of the filament, upon which both terminal regions of the molecule become well folded, forming alpha-helical bundles with their axes parallel to the filament axis.

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